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Interrelationships between Energy Metabolism, Growth Dynamics, and Reproduction during the Life Cycle of 'Mysidopsis bahia' as Influenced by Sublethal Endrin Exposure

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16. ABSTRACT

The estuarine myside, Mysidopsis bahia, was reared through an entire life-cycle in a range of endrin concentrations. Physiological rates of ammonia excretion and oxygen consumption were measured for various life stages. These individual functions and those of growth and reproduction were combined into bioenergetic and physiological indices to help describe the chronic effects of endrin on mysids. Concentrations of endrin that were chronically lethal stimulated the respiration rates of newly released juveniles after only one day exposure. Sublethal exposure to endrin reduced growth rates of rapidly growing juveniles. In accordance with lower net growth efficiences (K_2 values), increased metabolic demands accompanying endrin exposure allowed for less of the assimilated energy being available for production of new tissue. Energy metabolism from release of brooded juveniles until maturation of adults was partially based on lipid utilization. During maturation increased amounts of protein were used as an energy source, as indicated by higher ammonia excretion rates and lower O:N ratios. Endrin inhibition of reproduction was more a product of fewer females becoming reproductively active, than simply reduced fecundity of individual females. Lessened reproductive abilities with sublethal endrin exposure correlated with increased lipid catabolism during maturation. It is postulated that enhanced energy demands, associated with higher metabolic rates during endrin exposure, favored usage of energy-rich lipid material while shunting lipids away from gametogenesis and reproduction.

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INTERRELATIONSHIPS BETWEEN ENERGY METABOLISM, GROWTH DYNAMICS, AND REPRODUCTION DURING THE LIFE CYCLE OF MYSIDOPSIS BAHIA AS INFLUENCED BY SUBLETHAL ENDRIN EXPOSURE

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INTRODUCTION

While information regarding lethality of potential environmental contaminants may aid in a comparative hazard assessment, greater ecological concern exists for the consequences of chronic exposure to microcontaminants. Relatively simple chronic-toxicity tests can provide information on such indicators of toxicant-induced biological damage, as survival, growth, and reproduction. More sophisticated exposure-effects studies may provide additional evidence of changes in vital life processes, including the site of action, and thus offer insight into mechanisms of pollutant toxicity. Since sublethal responses to pollutants by individual organisms occur and are observed before damage is demonstrable at the population, community, or ecosystem levels; biochemical and physiological measurements of an organism's "fitness" may serve as an early-warning of deterioration in water quality (NAS, 1971; Sprague, 1971; Waldichuk, 1979; Kinne, 1980; Sastry and Miller, 1981).

Summarizing papers presented at a recent international symposium, Kinne (1980) concluded that pesticides and related organic compounds represent the greatest threat to marine biota. Considering the close phylogenetic relationship between crustaceans and the insect pests for which pesticides are directed, it is not surprising that crustaceans often are more sensitive to pesticides than other marine organisms (Schimmel et al., 1975; Schimmel et al., 1976, 1977; Schimmel et al., 1979; Nimmo et al., 1981).

For the majority of pesticides examined in life-cycle toxicity tests using the estuarine crustacean, Mysidopsis bahia, a sublethal reduction in reproductive potential has proved to be the most sensitive criterion yet determined for chronic biological effects (Nimmo et al., 1977, 1979, 1980, 1981).

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From a bioenergetic point of view (Ivlev, 1945; Winberg, 1956, 1971; Klekowski and Duncan, 1975), the reproductive capacity of an organism is intimately associated with growth and metabolism. Of the total energy contained within the food of an individual, only that portion which is assimilated by the organism is "physiological useful energy". This assimilated energy is further partitioned into energy required for metabolic maintenance and energy shunted into production, including somatic growth and reproduction.

The purpose of this research was to determine: (1) chronically lethal concentrations of the chlorinated pesticide, endrin, for the estuarine mysid, Mysidopsis bahia, exposed through a complete life cycle; (2) if sublethal concentrations of endrin alter the reproductive capacity of exposed mysids; and (3) the influence of chronic sublethal exposure to endrin on various bioenergetic components in developing M. bahia. Energy metabolism for the different life stages during endrin exposure was investigated by measuring physiological rates of oxygen consumption and ammonia excretion. Individual rates of metabolism and growth during particular life stages were combined into indices to help understand the chronic effects of endrin on estuarine mysids and to describe physiological mechanisms of endrin toxicity in an estuarine crustacean.

MATERIALS AND METHODS

Exposure System

Mysids were exposed to endrin in a flow-through exposure system with the various endrin stock solutions and the carrier-control triethylene gylcol (TEG) continuously introduced by an infusion pump into constantly flowing seawater maintained at 20 ± 2°/$_{oo}$ S and 25 ± 1°C. The exposure medium was delivered through an indented glass mixing-tube into a glass splitting-box which emptied into a glass aquarium divided into separate replicate chambers.

Concentrations of endrin in the exposure water were detected by electron-capture gas chromatography using a 182 cm x 2 mm i.d. glass column packed with 2% OV-101 on 100-120 mesh Gas Chrom Q. The nitrogen flow rate was 25 ml/min; oven temperature, 190°C; and injector and detector temperatures, 210°C. Recovery of endrin-spiked water samples exceeded 85%; data were not adjusted for recovery.

Experimental Design

A preliminary experiment was performed in which 4 replicates of 20 mysids each were exposed to measured endrin concentrations of 0 (TEG control), 120, 250, and 570 ng/l. Oxygen consumption measurements were made on mysids from 1 replicate after 24 h. Survival observations were made on mysids in the remaining 3 replicates on d 4 of exposure.

The main experiment was a 4x6 factorial design utilizing 4 different life stages and 6 exposure concentrations. Measured endrin concentrations were 0 (TEG control), 7, 15, 30, 61, and 108 ng/l. Survival observations and metabolic/growth measurements were made at each exposure concentration on 4 life stages: early juveniles (d 1), juveniles (d 4), advanced juveniles (d 10), and young adults (d 16).

Collection and Maintenance of Experimental Animals

Newly released juvenile Mysidopsis bahia (<24 h old) were obtained from ovigerous females maintained in static, recirculating cultures at 20 ± 2‰ S and 25 ± 1°C. For the survival/reproduction portion of the study, 20 juveniles were pipetted into Nitex® baskets in each of 3 replicate chambers. Additional groups of mysids were placed in separate baskets at each exposure concentration and subsampled for metabolic/growth measurements. Throughout the study, all groups of mysids were fed an abundance of freshly hatched Artemia nauplii daily.

Survival and Reproduction

On d 1, 4, 10, and 16, observations were made on survival in each of 3 replicate groups of 20 mysids exposed to the various endrin concentrations. Commencing on d 16, mysids were observed daily for ovigerous females and these females were isolated in pairs with mature males within Nitex® brood cups. Since most female marsupia appeared before d 16, pairing could have been performed earlier. However, pairing after eggs were deposited best allowed for natural reproductive behavior among the entire group of mysids as would occur in a field population and prevented the pairing of 2 females

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if the appearance of the marsupium of 1 was delayed. Daily records were kept on survival and number of young released in the first broood by isolated females. Past experience has shown that toxicant-induced delay in release of young occurs within 1 wk (McKenney, unpublished observations). Hence, the study was terminated 1 wk after the mean day of release of the first brood by control mysids.

Metabolic Measurements

On d 1 of the preliminary experiment and on d 1, 4, 10, and 16 of the main experiment, 5 mysids from each exposure concentration were sealed individually in 5-ml all-glass syringes containing 1 to 3 ml (dependent on the size of the mysid) of fully oxygenated media at the appropriate endrin concentration. Syringes containing mysids were incubated in a 25°C water bath along with control syringes without mysids and syringes containing ammonia standards. After 4 to 6 h, a 75-100 µl sample of the fluid in the syringe was injected into a Radiometer BMS3 Mk2 Blood Micro System with attached PHM71 Mk2 Acid-Base Analyzer and a direct measurement recorded for partial pressure of oxygen. In addition, a 100 µl sample of the fluid was injected into a micro-flow-through cap (Model 95-00-25) attached to an Orion Ammonia Electrode (Model 95-10) connected to a Radiometer PHM64 Research pH Meter. Millivolt readings were converted to ammonia concentrations with a calibration curve produced from ammonia standards of known concentrations. Each mysid was then removed from the respirometer, briefly rinsed in distilled water, and placed in an oven at 60°C to dry for 48 h. Dry weights of individual mysids were subsequently measured to the nearest 0.1 µg on a Cahn 21 Automatic Electrobalance.

Differences between partial pressures of oxygen and ammonia concentrations in control respirometers (syringes with no mysid) and respirometers containing a mysid were attributed to mysid respiration and ammonia excretion, respectively. Both weight-specific oxygen consumption rates (µg O_2 mg^{-1} dry weight h^{-1}) and weight-specific ammonia excretion rates (µg NH_3-N mg^{-1} dry weight h^{-1}) were calculated. O:N ratios were calculated as the ratio of atoms of oxygen consumed to atoms of nitrogen excreted.

Growth

Several growth indices were calculated from the dry weights of each mysid life stage exposed to the various endrin concentrations by the formulas of Winberg (1971). The mean specific rate of growth was calculated thus:

$$g_n = \frac{(\ln W_{n+1} - \ln W_n)}{t} \quad (1)$$

where g_n = the mean specific rate of growth of a particular life stage n, W_{n+1} = the mean dry weight of life stage n+1, W_n = the mean dry weight of life stage n, and t = the number of days between life stage n and life stage n+1. The mean specific rate of growth was incorporated into the formula

$$G_n = (e^{g_n} - 1) \quad (2)$$

to quantify the fractional daily weight gain (G_n) for each life stage under the various endrin exposure concentrations. G_n was multiplied by the replicate dry weights to produce values of daily weight gain (WG) ($\mu g\ day^{-1}$) for each life stage-concentration combination.

Bioenergetic Terms

Daily production energy for each life stage under various endrin exposure conditions was determined by the formula

$$P_n = (WG_n \times TC) + 0.10\ (WG_n \times TC) \quad (3)$$

where P_n = gross daily production in joules for life stage n, WG_n = daily gain ($\mu g\ day^{-1}$) for life stage n, TC = the mean energy content for dry tissue of an estuarine mysid from Florida (18.598×10^{-3} joules per µg dry weight) (Johnson and Hopkins, 1978), and 0.10 ($WG_n \times TC$) = a correction for the average energy loss from marine crustaceans by shedding of exuviae (Lasker, 1966; Ivleva, 1970; Tiskhon-Lukanina and Lukasheva, 1970; Clutter and Theilacker, 1971; Mootz and Epifanio, 1974; Nelson et al., 1977; Logan and Epifanio, 1978).

Daily maintenance energy for each life stage was derived from the formula

$$R_n = VO_{2_n} \times 24 \times W_n \times OC \quad (4)$$

where R_n = daily expenditure in joules for maintenance during life stage n, VO_{2n} = the respiratory rate ($\mu g\ O_2\ mg^{-1}$ dry weight h^{-1}) for life stage n, W_n = dry weight (mg) of life stage n, and OC = the oxycalorific coefficient in joules for carnivores that utilize ammonia as their chief excretory product (13.556×10^{-3} joules per µg O_2) (Elliott and Davison, 1975).

Net growth efficiency (K_2) (Ivlev, 1945; Winberg, 1956, 1971; Calow, 1977) was derived for each life stage under each endrin exposure condition by the formula

$$K_{2_n} = \frac{P_n}{(P_n + R_n)} \quad (5)$$

where K_{2_n} = the net growth efficiency for life stage n, P_n = the daily production in joules for life stage n, and R_n = the daily expenditure in joules for maintenance for life stage n.

Statistical Analysis and Development of Response Surface Curves

Differences in biological responses between endrin concentrations were analyzed by an analysis of variance, using Dunnett's procedure for multiple comparisons between treatment means and the control mean (Steel and Torrie, 1960; Sokal and Rohlf, 1969). Relationships between biological responses and endrin concentrations were examined by linear regression analysis, while more complex relationships between biological responses, endrin concentrations, and mysid life stages were examined by multiple regression analysis (McNeil et al., 1975; Box et al., 1978; Draper and Smith, 1981).

The most complex model was a two-factor, second-order polynomial equation, in which the entire data set was regressed on the quadratic function of life stage and endrin concentration:

$$\hat{Y} = b_0x_0 + b_1x_1 + b_{11}x_1^2 + b_2x_2 + b_{22}x_2^2 + b_{12}x_1x_2 \quad (6)$$

where $\hat{Y}$ = the estimated response; x_0 = the intercept; x_1 and x_2 = the linear effect of endrin concentration (Endrin) and life stage of mysid (Stage), respectively; x_1^2 and x_1^2 = the quadratic effect of endrin concentration ($Endrin^2$) and life stage ($Stage^2$), respectively; x_1x_2 = the linear x linear interaction between endrin concentration and life stage (Endrin X Stage); and b_0, b_1, etc. = the regression coefficients (Box, 1954; Box and Youle, 1955; Myers, 1971; Alderdice, 1972). If this model best explained the data, response surface curves were generated at a range of levels of these two factors by the General Linear Models (GLM) and PLOT procedures available in the SAS statistical package (Barr et al., 1979).

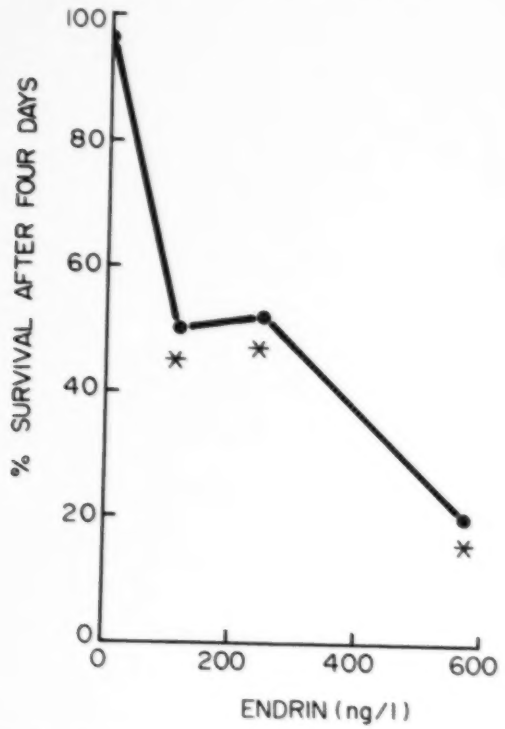


Fig. 1. Acute lethality of Mysidopsis bahia juveniles as a function of endrin exposure for 4 d. Asterisks denote values significantly different from control at .05 probability level.

RESULTS

The results of a preliminary experiment showed significant reductions in survival of juvenile Mysidopsis bahia after a 4-d exposure to endrin concentrations above 120 ng/l (Fig. 1). Linear regression analysis demonstrated a direct increase in the oxygen consumption rates of juvenile mysids as a function of exposure to acutely toxic endrin concentrations after only 1 d exposure ($R^2 = 0.725$) (Fig. 2).

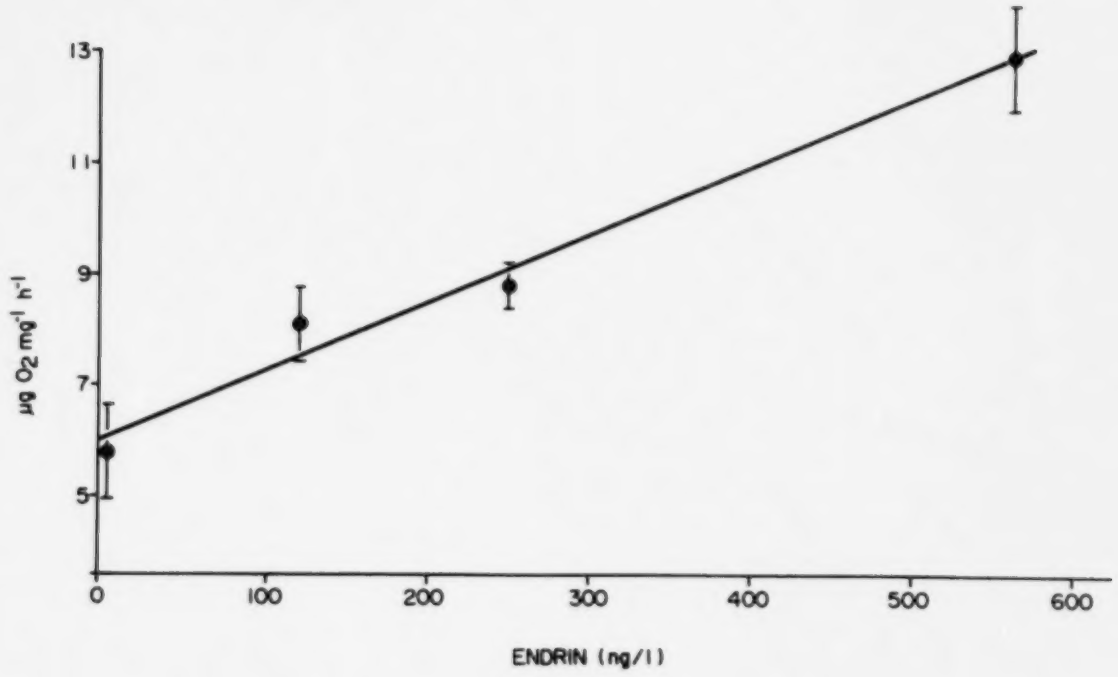


Figure 2.

Survival of newly-hatched juveniles to adulthood and successful release of the first brood was reduced by exposure to progressively higher levels of endrin (Fig. 3). Dunnett's procedure showed these reductions in mysid survival through a complete life cycle to be significantly different from unexposed controls at exposure concentrations of 60 ng/l endrin and higher.

As with exposure to acutely toxic concentrations of endrin, exposure to chronically lethal concentrations resulted in elevated oxygen consumption rates of mysids (Fig. 4). A two-way analysis of variance of the oxygen consumption data (Table 1) showed that respiration not only varied significantly with endrin exposure, but also varied with life stage. Highest weight-specific oxygen consumption rates of control M. bahia occurred in first-day juveniles, and the rates decreased with age. Linear regression of respiration data by life stage produced R^2 values lower than 0.30, suggesting less of a direct relationship between chronically lethal and sublethal endrin concentrations and mysid respiration than had existed for acutely toxic concentrations. Nevertheless, Dunnett's procedure demonstrated a significant increase in the oxygen consumption rates of advanced juveniles (d 10) exposed to 108 ng/l endrin and significantly higher oxygen consumption values for pooled juvenile mysid data at the exposure concentration of 60 ng/l endrin.

Analysis of variance of the daily growth rates of endrin-exposed mysids demonstrated a highly significant ($p \leq .001$) influence of both endrin and life stage on somatic production, in addition to a significant interaction between these two factors. To explore these relationships further, multiple linear regression analysis was performed on the growth data. A model utilizing the quadratic function of endrin concentration and life stage explained 82% of the variation in growth data (Table 2). Mysid growth rates were most influence by their stage in the life cycle, while also being significantly altered by both endrin exposure and a stage-endrin interaction. To best illustrate these relationships, response surface curves were developed from the coefficients of this model (Fig. 5). As shown by the intensity shading of the isopleths, daily growth rates progressively increased through the juvenile period to a maximum growth rate of more than 70

Fig. 2. Linear regression of oxygen consumption rates of Mysidopsis bahia juveniles as a function of endrin exposure for 1 d. Values represent means ± SE.

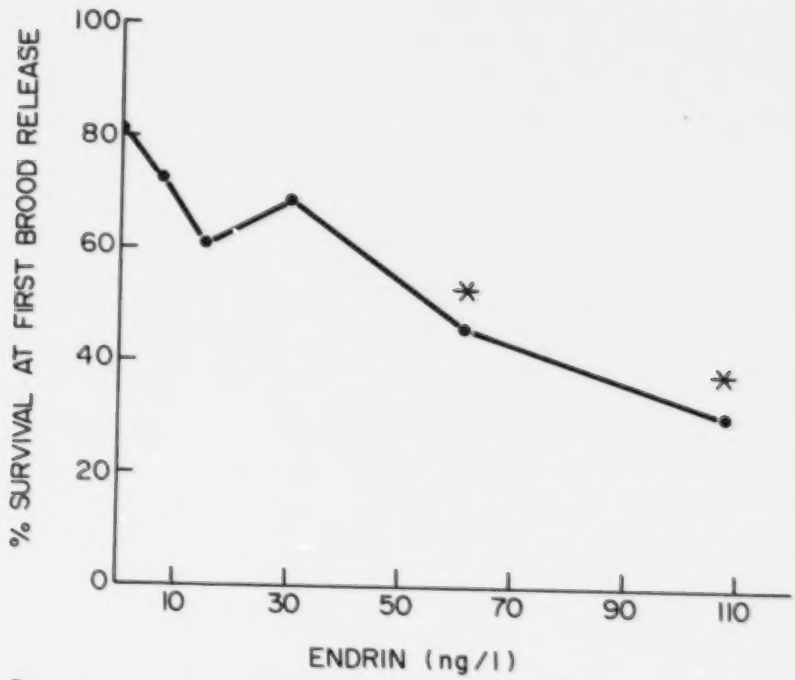


Fig. 3. Chronic lethality of Mysidopsis bahia as a function of endrin exposure through an entire life cycle. Asterisks denote values significantly different from control at .05 probability level.

$\mu g\ day^{-1}$ for advanced juveniles (d 9-10), then diminished as juveniles approached maturity (d 12-18). Endrin exposure most influenced the growth rates of rapidly growing juveniles. Dunnett's procedure demonstrated significant reductions in growth rates from d 1 to d 4 at endrin concentrations of 60 and 108 ng/l and from d 4 to d 10 at 60 ng/l.

Net growth efficiencies (K_2 values) were also examined by multiple linear regression analysis. As with growth rates, the quadratic function of life stage and endrin exposure best explained the data ($R^2 = 0.922$) (Table 3). Response surface curves (Fig. 6), illustrate the highly significant influence of life stage, endrin exposure, and interactions between these two factors on K_2 values. Net growth efficiency was significantly higher for the juvenile stages (d 4 and d 10) than for adults. Endrin exposure most influ-

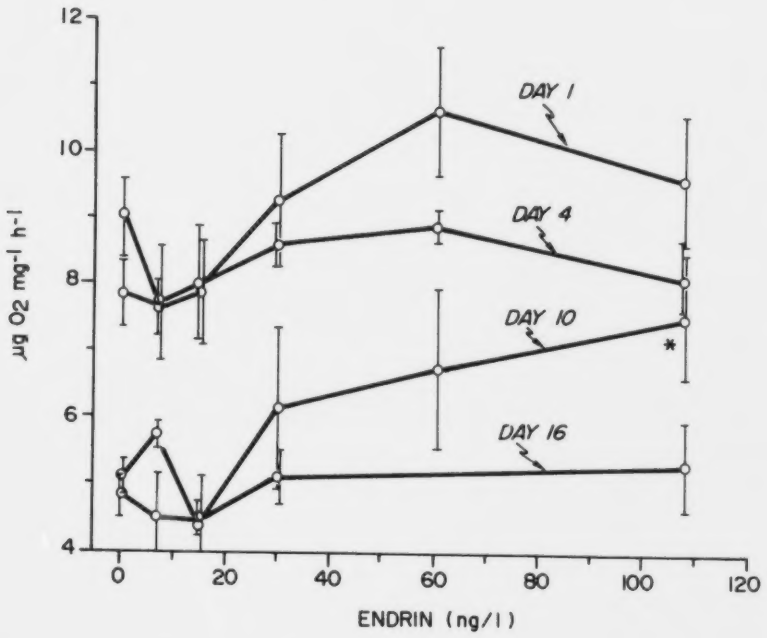


Fig. 4. Oxygen consumption rates of early juvenile (d 1), juvenile (d 4), advanced juvenile (d 10), and adult (d 16) Mysidopsis bahia as a function of endrin exposure through an entire life cycle. Values represent means ± SE. Asterisks denote values significantly different from control at 0.5 probability level.

enced net growth efficiencies of juveniles, significantly reducing K_2 values of 4-d old juveniles at concentrations above 30 ng/l.

The effects of chronic endrin exposure on reproduction of Mysidopsis bahia were evaluated by several criteria. The number of young released per female in the first brood was significantly reduced by exposure to concentrations which eventually proved to be chronically lethal (Fig. 7A).

Table 1. Two-way analysis of variance of the weight-specific oxygen consumption rates of Mysidopsis bahia as influenced by stage in life cycle and continuous exposure to endrin from 7 to 108 ng/1.

Source of variation	DF	MS	F-value	p-value
Total	113	5.67400		
Treatments	22	16.89316		
Stage	3	91.80997	31.00	.0001
Endrin	5	15.06694	5.09	.0004
Stage X Endrin	14	1.49178	0.50	.9255
Error	91	2.96168		

Table 2. Multiple linear regression analysis of the growth rates (µg day-1) of Mysidopsis bahia as influenced by stage in life cycle and continuous exposure to endrin from 7 to 108 ng/1.

Source of variation	DF	MS	F-value	p-value
Total	85	538.63		
Model	5	7464.93	70.60	.0001
Endrin	1	1803.02	17.05	.0001
$Endrin^2$	1	535.91	5.07	.0271
Stage	1	32119.54	303.76	.0001
$Stage^2$	1	35287.45	333.72	.0001
Endrin X Stage	1	1206.39	11.41	.0011
Error	80	105.74		

Although the male:female ratio at maturity did not differ among the treatment populations, the total number of offspring produced was inversely related to the exposure concentration (Fig. 7B). The total progeny released by a discrete population of mysids was significantly lower with sublethal exposure above 15 ng/l endrin. By isolating mysid females individually at maturity, the percentage of females contributing offspring to the population could be determined. This criterion proved to be the most sensitive measurement of endrin's effect on mysid reproduction. The percentage of females that became reproductively active after continuous endrin exposure since d 1 was inversely related to the exposure concentration and a significantly smaller percentage of females produced viable young in endrin concentrations above 7 ng/l (Fig. 7C).

A two-way analysis of variance demonstrated that ammonia excretion of M. bahia was significantly influenced by stage in life cycle, endrin exposure, and a stage-endrin interaction (Table 4). For unexposed mysids, ammonia excretion rates were lowest during juvenile and advanced juvenile stages (d 4 and d 10) and shifted toward significantly higher excretion rates for adults on d 16 (Fig. 8). Exposure to endrin modified this developmental pattern and, as indicated by the significant interaction term, these endrin-induced metabolic alterations were dependent on life stage. After 1 d of exposure to endrin, the lowest concentration (7 ng/l) resulted in a significant reduction in ammonia excretion rates of juveniles. Following 4 d of exposure, the 2 lowest concentrations produced similar reduction in ammonia excretion rates. The elevation of ammonia excretion rates observed in control juvenile mysids as they approached maturity was significantly depressed in mysids exposed to endrin concentrations ranging from 7 to 108 ng/l (Fig. 8).

Relationships between amounts of oxygen consumed and nitrogen released as ammonia as a result of catabolic processes were examined by computing O:N ratios. Like ammonia excretion rates, O:N ratios of Mysidopsis bahia exposed to endrin were modified by life stage, endrin exposure, and a stage-endrin interaction (Table 5). O:N ratios of unexposed mysids were highest during juvenile stages (d 4 and d 10) and were dramatically reduced as adults (Fig. 9). Progessively higher sublethal concentrations of endrin resulted in elevated O:N ratios during the first 4 d as juveniles. Exposure to endrin significantly increased the low O:N values of adults at concentrations above 15 ng/l.

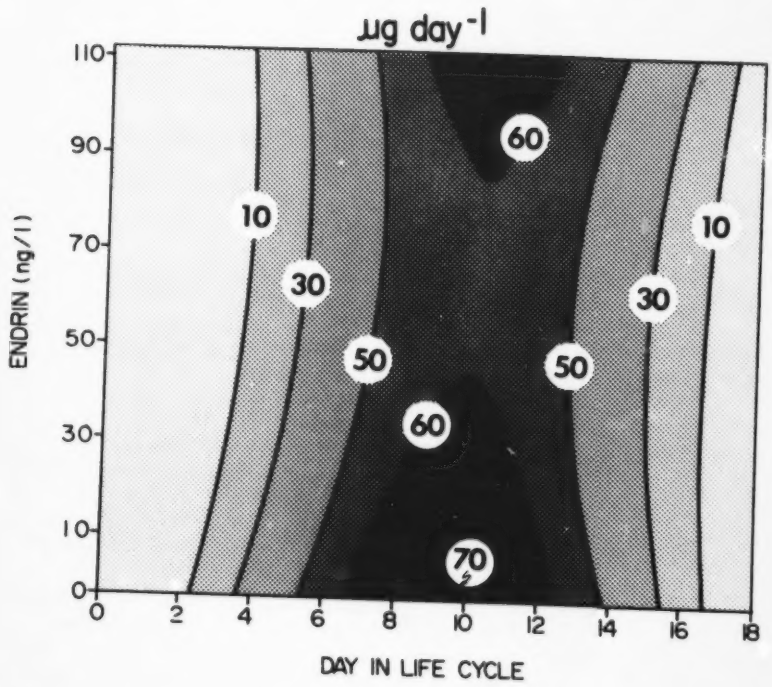


Fig. 5. Response surface of growth rates (µg day^{-1}) of Mysidopsis bahia as influenced by continuous endrin exposure through an entire life cycle.

DISCUSSION

The life history of an organism consists of three basic interrelated biological components: maintenance, growth, and reproduction. During the life cycle of an organisms, internal physiological and biochemical processes must be coordinated to allow the proper apportionment of available energy to satisfy these individual energy-demanding life functions.

For organisms utilizing oxidative metabolism, rates of oxygen consumption may be used to measure the total energy required in the metabolic reactions necessary for an organism's maintenance. Oxygen consumption rates of the estuarine mysid, Mysidopsis bahia, varied with life stage from the high value of 9.1 µg O_2 $mg^{-1} \cdot h^{-1}$ for 1-d old juveniles to 7.8 µg O_2 $mg^{-1} \cdot h^{-1}$ for 4-d old juveniles to

Table 3. Multiple linear regression analysis of the net growth efficiencies (K_2 values) of Mysidopsis bahia as influenced by stage in life cycle and continuous exposure to endrin from 7 to 108 ng/l.

Source of variation	DF	MS	F-value	p-value
Total	82	249.160		
Model	5	3439.587	81.91	.0001
Endrin	1	296.742	7.07	.0095
Endrin^2	1	5.789	0.14	.7114
Stage	1	2396.077	57.06	.0001
Stage^2	1	5533.113	131.77	.0001
Endrin X Stage	1	771.237	18.37	.0001
Error	77	41.990		

approximately 5 μg O_2 $mg^{-1} \cdot h^{-1}$ for advanced juveniles and mature adults. This inverse relationship between weight-specific oxygen consumption rates and age has also been described for other littoral mysids, e.g., Metamysidopsis elongata (Clutter and Theilacker, 1971) and Gastrosaccus psammodytes (Dye, 1980). Similar reductions in metabolic rates through progressively older life stages have been described in marine copepods (Conover and Corner, 1968) and in decapod crustaceans (Mootz and Epifanio, 1974; McKenney and Neff, 1981). These variations in metabolic rates with age may be due to changes in tissue types having differing metabolic demands (Prosser, 1973) or may be a product of various substrates being utilized during different life stages, as shown in other crustaceans, including both copepods (Conover and Corner, 1968) and decapods (Capuzzo and Lancaster, 1979; McKenney, 1979).

Concentrations of the chlorinated insecticide, endrin, that were lethal to Mysidopsis bahia in a 4-d period stimulated oxygen consumption rates after only 24 h of exposure. Metabolic rates of mysids were also elevated by exposure to subacute endrin concentrations throughout their life cycle, particularly at concentrations that were chronically lethal. Similarly, high subacute internal concentrations of the chlorinated insecticides, Mirex and DDT, in

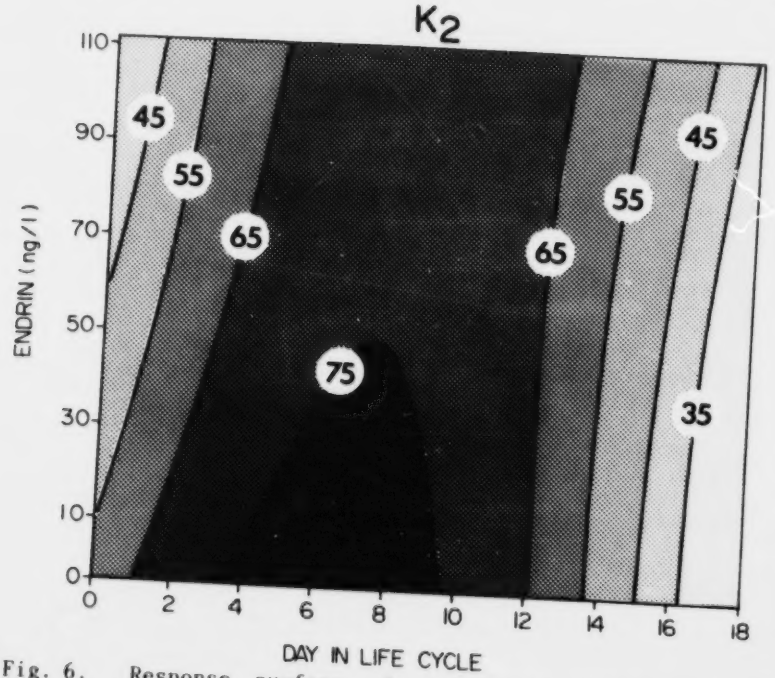
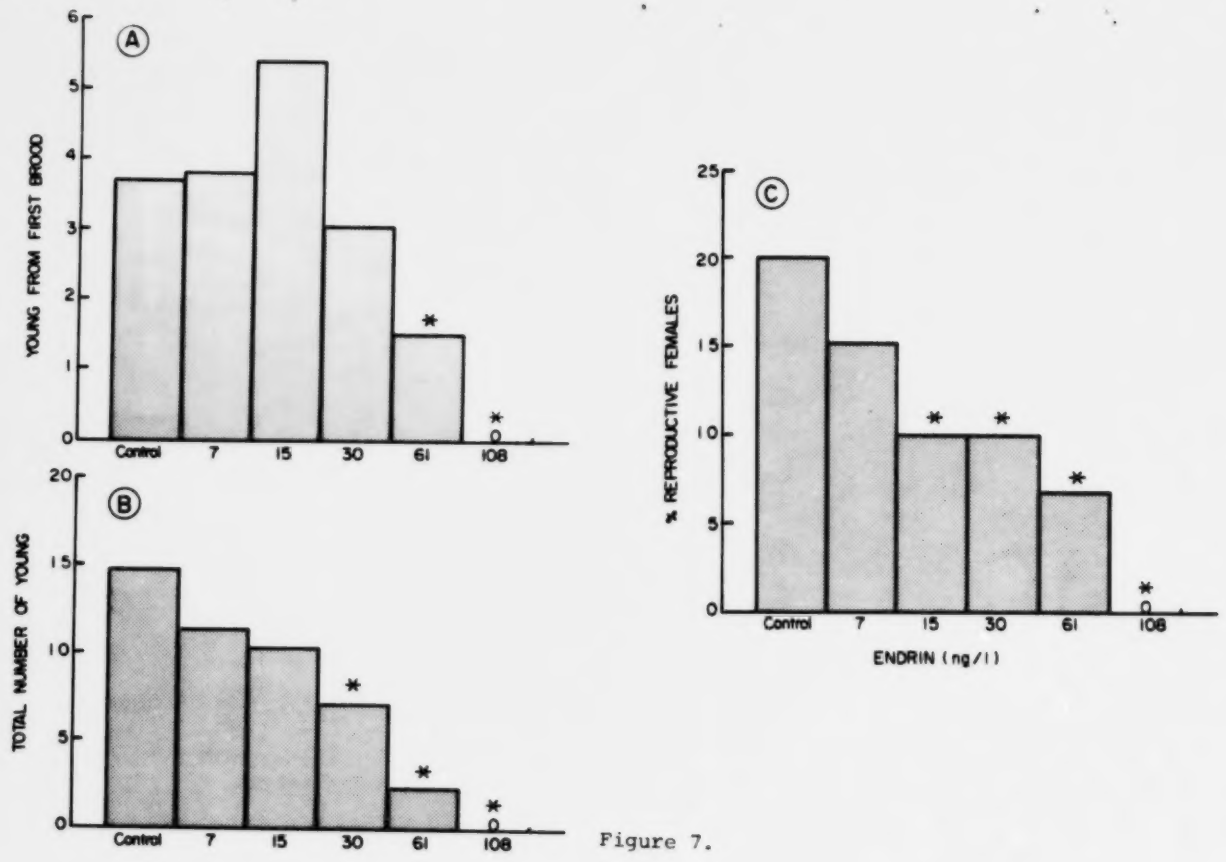


Fig. 6. Response surface of net growth efficiencies (K_2 values) of Mysidopsis bahia as influenced by continuous endrin exposure through an entire life cycle.

juvenile blue crabs, Callinectes sapidus, increased rates of oxygen uptake (Leffler, 1975). Acute sublethal exposure to chlorinated organics elevated oxygen consumption rates in juvenile horseshoe crabs (Neff and Giam, 1977) and adult fiddler crabs (Vernberg et al., 1978). Respiration of both a freshwater (Rao et al., 1980) and a marine fish (Anderson,

Fig. 7. Reproductive performance of Mysidopsis bahia as a function of endrin exposure through an entire life cycle. Responses include: (A) number of young released per female in the first brood; (B) total number of young produced in first brood by initial population of 20 one-day-old juveniles; and (C) percentage of reproductively active females in the population. Asterisks denote values significantly different from control at .05 probability level.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% REPRODUCTIVE FEMALES
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25
ENDRIN (ng/l)

Figure 7.

Table 4. Two-way analysis of variance of the weight-specific ammonia excretion rates of Mysidopsis bahia as influenced by stage in life cycle and continuous exposure to endrin from 108 ng/l.

Source of variation	DF	MS	F-value	p-value
Total	92	0.024312		
Treatments	22	0.075380	9.12	.0001
Stage	3	0.297255	35.98	.0001
Endrin	5	0.046651	5.65	.0002
Stage X Endrin	14	0.038095	4.61	.0001
Error	70	0.008262		

1971) was increased by sublethal exposure to organochlorine insecticides. Observations of increased respiration in insects treated with cyclodiene insecticides, like endrin, suggested that elevated respiration rates that accompany acute exposure may be associated with hyperactivity, whereas elevated metabolic rates during chronic exposure may best be related to interference with mitochondrial enzymes and the uncoupling of oxidative phosphorylation, thereby decreasing the efficiency of energy utilization and inducing the organism to respire more (Brooks, 1974; Wilkinson, 1976).

Growth rates of M. bahia increased from marsupial release through the juvenile period to a maximum rate of somatic production during advanced juvenile stages. As juveniles developed to maturity, their growth rate diminished. A response surface of daily weight gain indicated that sublethal exposure to endrin selectively retarded tissue production in rapidly growing juvenile mysids, while not altering growth rates in early juveniles or maturing adults. Similarly, growth of juvenile cockles was retarded with sublethal exposure to the insecticide Sevin (Butler et al., 1968). Chronic exposure of sexually mature teleost fish to endrin suppressed normal activity of the thyroid gland, thought to function in regulation of cellular metabolism, causing lowered growth rates (Grant and Mehrle, 1970).

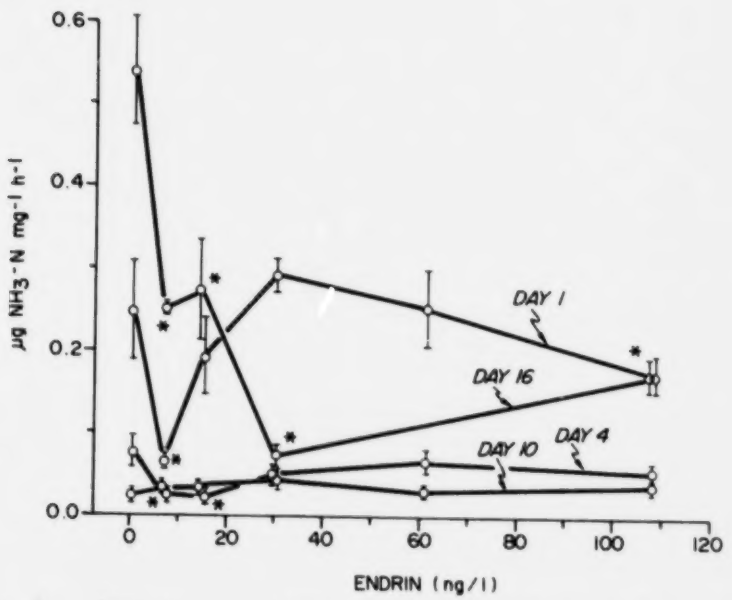

Fig. 8. Ammonia excretion rates of early juvenile (d 1), juvenile (d 4), advanced juvenile (d 10), and adult (d 16) Mysidopsis bahia as a function of endrin exposure through an entire life cycle. Values represent means ± SE. Asterisks denote values significantly different from control at .05 probability level.

Tissue production is intimately associated with the organism's metabolic maintenance demands. By combining caloric equivalents for individually derived rate functions of both oxygen consumption and growth into bioenergetic formulas, net growth efficiencies (K_2 values) can be calculated to estimate the conversion efficiency of assimilated energy either into growth or into energy necessary for metabolic maintenance. Disparities in the ratio of assimilated energy available for growth may serve as useful indicators of physiological stress. High growth rates of M. bahia juveniles were a result of more energy being channeled into tissue production relative to that required for maintenance. Maintenance requirements increased for juvenile mysids stressed by sublethal endrin exposure, as suggested by the elevated respi-

Table 5. Two-way analysis of variance of O:N ratios of Mysidopsis bahia as influenced by stage in life cycle and continuous exposure to endrin from 7 to 108 ng/l.

Source of variation	DF	MS	F-value	p-value
Total	89	7966.18		
Treatments	22	24653.24	9.91	.0001
Stage	3	129182.36	51.95	.0001
Endrin	5	10414.31	4.19	.0024
Stage X Endrin	14	7339.47	2.95	.0015
Error	67	2486.85		

ration rates, and less energy remained for tissue production. A related estuarine mysid, Mysiodopsis almyra, responded similarly during sublethal exposure to aqueous fractions of drilling muds. Even though juveniles exposed to mud aqueous fractions consumed more food, higher respiration rates were accompanied by stunted growth (Carr et al., 1980).

As organisms mature, energy demands become necessary for development of functional gonads and production of viable gametes. One would expect that metabolic processes associated with these events, involving energy mobilization and storage, would be influenced by bioenergetic imbalances resulting from physiological stress. Exposure to chronically lethal concentrations of endrin caused individual M. bahia females to produce fewer young and a smaller percentage of females in the population to become reproductively active and contribute young to the population. As a result, mysid populations exposed during their life cycle released fewer total offspring. Similar reductions in reproductive potential of mysids have been reported with sublethal exposure to other pesticides (Nimmo et al., 1977, 1979, 1980, 1981). This reproductive impairment would result in diminution of mysid populations which could directly affect fish populations that

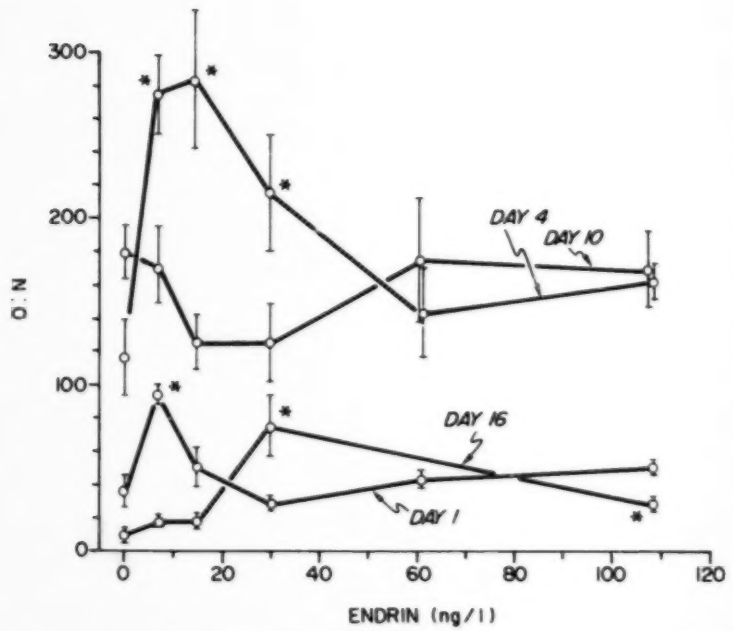

Fig. 9. Atomic ratio of oxygen consumed to ammonia-nitrogen excreted for early juvenile (d 1), juvenile (d 4), advanced juvenile (d 10), and adult (d 16) Mysidopsis bahia as a function of endrin exposure through an entire life cycle. Values represent means ± SE. Asterisks denote values significantly different from control at .05 probability level.

depend upon mysids as a food source and could disrupt the delicate balance of estuarine and marine food webs (Darnell, 1958; Carr and Adams, 1973; Adams, 1976; Livingston, 1980).

The O:N ratio is an index of the portion of total oxidative metabolism that is due to protein catabolism. Minimal O:N ratios of 4 to 8 for planktonic marine crustaceans represent theoretical values for pure protein catabolism (Conover and Corner, 1968; Mayzaud, 1973). O:N ratios of 11 to 22 for the adults of several shallow-water marine mysids (Chin, 1974; Gaudy et al., 1980) suggest that protein is a major substrate

for oxidative metabolism in these organisms. Furthermore, biochemical analysis of nearshore marine and estuarine mysid adults showed 55-80% protein; the remaining portion, excluding chitin and ash, was principally lipid with a trace amount (2% or less) of carbohydrates (Raymont et al., 1968; Clutter and Theilacker, 1971; Johnson and Hopkins, 1978). Since Artemia also contain only minimal amount of carbohydrates (Benijts et al., 1975), alterations in the O:N ratios of Mysidopsis bahia in this study should reflect relative changes in protein or lipid usage as metabolic substrates.

O:N ratios of 169-179 for M. bahia juveniles suggest that growing mysid juveniles utilize lipid substrates as their principal metabolic energy source. As juveniles mature, ammonia excretion rates increase and O:N ratios are lowered, indicating a metabolic shift to increased protein substrate utilization. These metabolic shifts toward greater reliance on protein catabolism as mysids mature could reflect the necessary mobilization and transfer of lipid material to developing gonads as a prerequisite for successful gametogenesis. Similar to these results nitrogen excretion was higher and O:N ratios lower for gravid marine copepods than for non-gravid individuals (Conover and Corner, 1968). Mobilization, storage, and utilization of lipid reserves for reproduction have been reported for other marine zooplankton with mature adults containing more lipids than immature adults (Lawrence, 1976; Gatten et al., 1979).

Higher O:N ratios observed in early juvenile mysids exposed to endrin suggest that added energy-demands due to pesticide exposure required greater use of the more energy-rich lipid materials to meet elevated maintenance costs. Sublethal exposure to water soluble fractions of petroleum hydrocarbons resulted in catabolism of stored lipids and significant decreases in percentage lipid of the marine amphipod, Amphithoe valida (Lee et al., 1981). Since ammonia excretion rates represent a measure of absolute amounts of protein being catabolized, the reduced ammonia excretion rates of maturing adults exposed to endrin could reflect augmented expenditure of metabolic energy reserves to meet higher metabolic demands accompanying endrin exposure and result in shunting of this lipid material away from necessary physiological and biochemical preparations for successful reproduction.

SUMMARY

The estuarine mysid, Mysidopsis bahia, was reared through an entire life cycle while exposed to a range of endrin concentrations. Alterations in survival and reproduction were

monitored during the exposure period. Energy metabolism of different life stages during endrin exposure was investigated by measuring physiological rates of oxygen consumption and ammonia excretion. These individual metabolic rate functions and those of growth were combined into bioenergetic and physiological indices to describe physiological mechanisms of endrin toxicity in an estuarine mysid.

Weight-specific oxygen consumption rates of M. bahia were inversely related to age during its life cycle. Concentrations of endrin that were acutely lethal stimulated respiration rates of newly released juveniles after only 1 d of exposure. Exposure to chronically lethal concentrations similarly resulted in higher metabolic rates, but to a lesser extent.

Growth rates of M. bahia increased through juvenile development to a maximum in advanced juvenile stages and diminished as juveniles developed to maturity. Sublethal exposure to endrin reduced growth rates of rapidly growing juveniles. In accordance with lower net growth efficiencies (K_2 values), increased metabolic demands accompanying endrin exposure reduced the amount of assimilated energy available for production of new tissue.

Energy metabolism during juvenile stages of M. bahia was based primarily on utilization of lipid substrates. During the maturation period, increased amount of protein were used as an energy source, as indicated by higher ammonia excretion rates and lower O:N ratios. Inhibition of mysid reproduction by endrin was a result of fewer females becoming reproductively active, rather than reduction in fecundity of individual females. Lessened reproductive capacity due to sublethal endrin exposure was correlated with increased lipid catabolism during maturation. It is postulated that enhanced energy demands, associated with higher metabolic rates during sublethal endrin exposure, favored usage of energy-rich lipid material at the cost of shunting lipids away from gametogenesis and preparation for reproduction.

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